

Non-SMC Condensin I Complex Subunit G Is a Prognostic Biomarker of Immune Infiltration in Non-small Cell Lung Cancer

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Abstract

Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths. Non-SMC condensin I complex subunit G (NCAPG) plays a significant role in tumor development. This study aimed to analyse the prognostic value and immunotherapy of NCAPG in non-small cell lung cancer. We set up a tissue microarray (containing 140 NSCLC and 10 normal lung tissues) and performed immunohistochemistry to assess NCAPG expression in the tissues of 140 patients. The receiver operating characteristic curves showed the diagnostic value of NCAPG. The prognostic value of NCAPG in NSCLC was assessed using the univariate and multivariate Cox proportional hazards regression models and Kaplan–Meier plots. We analyzed the association between NCAPG and immune infiltration in NSCLC. In addition, NCAPG expression and the degree of immune infiltration were evaluated based on data from TIMER and cumulative survival probability, and gene set enrichment analysis (GSEA) of NCAPG was performed. Based on the database analysis and immunohistochemistry, the NCAPG expression was upregulated in patients with lung cancer compared with para cancer controls ($p < 0.001$). Multifactorial analysis and Kaplan–Meier plots revealed that upregulation of NCAPG expression was an independent factor in the prognosis of NSCLC. Data from CIBERSORT showed a negative correlation between NCAPG and the expression of memory CD4T cells, CD8T cells, dendritic cells, macrophages, mast cells, and NK cells ($p < 0.001$). GSEA revealed that cell cycle, adhesion and proliferation were significantly enriched in samples with a high NCAPG expression. NCAPG is a novel biomarker of prognosis and is associated with immune cell infiltration in the tumor microenvironment. Thus, it can be a potential target in NSCLC treatment.

1 Introduction

Previous studies have reported that the incidence of lung cancer has significantly increased globally in recent years. Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancer cases[1]. NSCLC comprises lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC)[2]. Lung cancer is the most common cancer and is the leading cause of cancer-related deaths worldwide. The current study aimed to investigate novel and reliable biomarkers to improve the diagnosis and treatment of lung cancer[3].

To identify NSCLC cancer-related targets, we analyzed data showing overlapping differentially expressed genes from Cancer Genome Atlas (TCGA), a large-scale open database[4]. Results showed that non-SMC condensin I complex subunit G (NCAPG) was correlated with poor prognosis in NSCLC. NCAPG is a concentrated protein with a relative molecular weight of approximately 114 kDa and with 1015 amino acids and is located on chromosome 4p15.31[5]. It is responsible for the condensation and stabilization of chromosomes during meiosis and mitosis[6]. Moreover, it mainly reorganizes chromosomes into rod-shaped mitotic ones and ensures the separation of sister chromatids during cell division[7]. NCAPG expression increases in several cancer types and a high expression of this protein is correlated with a poor prognosis. Thus, NCAPG plays a vital role in carcinogenesis. Several studies have found that NCAPG is an oncogene in several cancer types. The upregulation of NCAPG promotes the migration, proliferation, and invasion of breast cancer cells[8]. In addition, it is associated with the development and progression

of numerous cancers, such as hepatocellular carcinoma, gastric, and endometrial[9]. The degree of immune cell infiltration in the tumor microenvironment (TME) plays an important role in tumorigenesis, progression, metastasis, and treatment resistance. Measuring the concentration of tumor-infiltrating immune cells (TIIC) in different tumor micro-environments[10]. Data from TIMER were used to assess tumor immune resources. Furthermore, these methods were applied to evaluate the association between NCAPG and immune cell infiltration. Notably, our preliminary studies revealed that NCAPG participates in controlling multiple pathways associated with immunity. However, to date, the immunological significance of NCAPG in NSCLC has not been validated.

2 Materials And Methods

2.1 NCAPG expression in TCGA

We downloaded the transcriptomic and clinical data of patients with LUAD and LUSC patients from TCGA, a large-scale data generation project that has significantly contributed to cancer systems biology and cancer genomics[11]. Next, the transcriptomic information containing 1013 NCAPGs was collected to investigate the differences in NCAPG expression in NSCLC.

2.2 Sample and tissue microarrays

To assess the prognostic value of NCAPG in non-small cell lung cancer, We obtained a tissue microarray (TMA) (HlugC120PT01) from Superbiotek (Shanghai, China), which consisted of 140 non-small cell lung cancer tissue samples and 10 normal lung tissues. All patients had undergone lung cancer resection in 2005. All patients (112 men and 28 women) had access to complete clinical information. We used 140 NSCLC tissues to investigate the association between NCAPG expression and the clinicopathological features of patients with NSCLC and the prognostic value of NCAPG. We determined the reasonable tumor stage for these patients based on the 2004 World Health Organization criteria and the International TNM classification[12]. The participants did not receive preoperative chemotherapy, radiotherapy, or biological therapy.

2.3 Immunohistochemical staining

NSCLC and paired normal paracancerous tissues were available. We used standard immunohistochemical procedures to detect NCAPG expression. Samples with a thickness of 0.4 μm were produced, dewaxed, rehydrated, and incubated with NCAPG antibody (1:200; Abcam, Cambridge, the UK) in a wet chamber at 4°C. After the removal of antibodies, samples were added to the sections with horseradish peroxidase-conjugated secondary antibodies. Next, diaminobenzidine (1:50) was added to stain the sections, which were re-stained with hematoxylin and dehydrated for viewing[13].

2.4 Immunohistochemical quantification analysis

Six random fields of view were selected from each section, and they were scored according to the degree of staining and percentage of stained cells. Two or more experienced pathologists performed the scoring

using a double-blinded method. The immunohistochemical evaluation results were as follows: PFKFB4 color intensity 1: weak, 2: moderate, and 3: strong; and percentage of positive cells, 0 points: < 5%, 1 point: 5%–25%, 2 points: 26%–50%, 3 points: 51%–75%, and 4 points: > 75%. The final quantification of each sample was the multiplication of the intensity of cytoplasmic staining and the area of positive cells, which were scored using the following scale: negative, 0–3 points; weakly positive, 4–6 points; moderately positive, 7–9 points; and strongly positive, 10–12 points[14].

2.5 Immune characterization analysis

The immune response to 22 NCAPGs in patients with NSCLC was assessed using data from CIBERSORT (<https://cibersort.stanford.edu/>), which included 22 immune cell types[15]. Thus, its relevance to survival and molecular subpopulations was assessed. To determine the prognostic impact of NCAPG on NSCLC based on immune cell infiltration, tumor-infiltrating immune cells were analyzed using data from TIMER[16].

2.6 Enrichment analysis of NCAPG

Enrichment analysis was performed to calculate the statistical significance of genes and the consistent differences between two biological states. To determine which pathways are enriched in each phenotype, GSEA used nominal p-values, false discovery rate, and normalized enrichment scores to detect the top gene-enriched pathways in both groups. For each analysis, we performed 1000 genome comparisons of replicates[17]. Next, we identified the phenotypic labels for NCAPG expression levels. In addition, to classify the enrichment pathways in each phenotype, we used standard p-values and normalized enrichment scores. Only false discovery rates of < 0.05 were considered remarkable[18].

2.7 Statistical analysis

GraphPad Prism (V9.0) and Statistical Package for the Social Sciences software version (V26.0) were used for statistical analysis and data generation[19]. The chi-square test was applied to analyze the correlation between NCAPG expression and clinical characteristics. The Cox proportional hazards regression models were used for univariate and multifactorial analyses. A p-value of < 0.05 was considered statistically significant. Survival analysis was performed using the Kaplan–Meier method. In addition, the receiver operating characteristic curves were plotted to identify the diagnostic value of NCAPG in NSCLC[20].

3 Results

3.1 Clinical data of patients

The mean age of the participants was 60 (26–78) years, and 53% of patients were aged > 60 years. Moreover, there was a high proportion of male participants (80%). The participants were followed-up from 2005 to 2013. By the end of the follow-up period, 84 patients had distant metastases and tumor recurrence, and 91 died.

3.2 Expression of NCAPG in NSCLC tissues

Immunohistochemistry was used to detect NCAPG expression in 140 pairs of NSCLC tissues, with highly phenotyped renal papillary cell carcinoma serving as a strong positive control and low-expressing paraneoplastic lung tissue as a negative control (Fig.1a-d). In addition, NCAPG expression in NSCLC tissues was analysed based on TCGA data. The results showed that NCAPG expression was upregulated in NSCLC tissue samples. We found that the NCAPG expression was higher in NSCLC, LUAD, and LUSC tissue samples than in adjacent normal tissue samples ($p < 0.001$, Fig. 2a–c).

3.3 Diagnostic value of NCAPG expression in NSCLC

We assessed the diagnostic value of NCAPG expression according to the receiver operating characteristic curves, with an area under the curve of 0.973 (95% confidence interval: 0.963–0.984; $p < 0.001$; Fig. 2d). Results revealed that NCAPG has good specificity and sensitivity for diagnosing NSCLC.

3.4 Association between a high NCAPG expression and poor prognosis

We used the median expression level as a classification criterion. Next, the patients were divided based on the expression of NCAPG and clinicopathological parameters. Moreover, the clinicopathological characteristics were compared. As shown in Table 1, there was no statistically significant correlation between NCAPG and age ($p = 0.866$), sex ($p = 0.833$), tumor site ($p = 0.865$), and other clinicopathological features. However, NCAPG expression was significantly correlated with tumour stage ($p < 0.001$) (Fig. 2e) and os events ($p = 0.007$) (Fig. 2f).

Cox regression analysis was performed to explore the factors affecting the survival time of patients with NSCLC. As shown in Table 2, based on the univariate analysis, NSCLC stage and NCAPG expression were significantly associated with survival time ($p < 0.05$). Multifactorial analysis revealed that NCAPG expression was a prognostic factor for survival outcome in NSCLC ($p = 0.008$), independent of the clinical factors. Therefore, a high NCAPG expression was associated with poor prognosis in patients with NSCLC. The Kaplan- Meier method was used to obtain survival curves to evaluate the correlation between NCAPG and NSCLC prognosis based on long-term follow-up data. Results showed that patients with NSCLC with a high NCAPG expression had shorter survival than those with a low NCAPG expression ($p < 0.001$, Fig. 2g). Further, the survival of patients with LUSC and LUAD who presented with a high NCAPG expression was shorter than that of patients with a low NCAPG expression ($p < 0.05$, Figs. 2h, i). Taken together, NCAPG could be an independent prognostic biomarker for poor prognosis in NSCLC.

3.5 Relationship between NCAPG expression and immune cell infiltration

NCAPG is a critical factor influencing the immune status of NSCLS. Next, we analyzed the correlation between NCAPG expression and various immune cells in NSCLC (Fig. 3a). Data from CIBERSORT were used to analyze the effect of NCAPG on the extent of immune-related cell infiltration. In total, 22 immune cells were analyzed in the study. The difference in the proportion of 22 NSCLC species in the tumor tissues between the high and low NCAPG expression groups was presented as the violin plot (Fig. 3b).

Subsequent analysis revealed that T cells, CD8T cells, dendritic cells, macrophages, mast cells, NK cells, and resting NK cells were negatively correlated with NCAPG expression ($p < 0.001$). Therefore, NCAPG expression was significantly correlated with the immune status of TME in NSCLC.

3.6 Analysis of the correlation between NCAPG expression and six types of infiltrating immune cells

In patients with LUSC and LUAD, we analyzed the relationship between NCAPG expression and six types of infiltrating immune cells, which were as follows: B cells, CD8 T cells, CD4 memory T cells, neutrophils, macrophages, and dendritic cells.

Data showed that NCAPG expression was correlated with tumor-filtering immune cells, B cells ($p < 0.0001$), CD8 T cells ($p < 0.0001$), CD4 memory T cells ($p < 0.0001$), neutrophils ($p < 0.0001$), macrophages ($p < 0.0001$), and activated-phase dendritic cells ($p < 0.0001$) (Fig. 4a). Therefore, NCAPG and its related genes were important in immune cell infiltration in tumor pathology.

We used the timer database, Kaplan–Meier evaluation of survival curves, and analyzed by log-rank test. Results showed that NCAPG expression affected the prognosis of LUSC and LUAD via immune cell infiltration. Dendritic cell ($p < 0.05$) and B-cell ($p < 0.001$) infiltration was significantly associated with NCAPG survival in LUAD (Fig. 4b). However, immune cell infiltration (CD4 T cells, B cells, dendritic cells, and macrophages) was not significantly correlated with NCAPG survival in LUSC. Therefore, B-cell and dendritic cell infiltration affected the survival outcomes of patients with LUAD. Thus, NCAPG was involved in the regulation of immune cell infiltration in LUAD.

3.7 Enrichment of gene sets in NCAPG expression phenotypes

GSEA was performed to explore the mechanisms of NCAPG in NSCLC. GSEA-based NCAPG-related signaling pathways were analyzed using TCGA datasets in the low and high NCAPG groups. We performed GO enrichment analysis and GSEA of NSCLC based on data from TCGA, which was significantly enriched in GO terms, including the components of mitotic nuclear division, mitotic spindle, microtubulin binding, cell cycle, humoral response, multivesicular body (Fig. 5a). Genes that were significantly associated with NCAPG are shown (Fig. 5b,c). In samples with a low NCAPG expression, B-cell and T-cell receptor signaling pathways were enriched, and this mechanism was associated with cancer progression (Fig. 5d-i). GSEA showed that cell cycle, proliferation, and adhesion-linked gene sets were enriched in samples with high NCAPG expression (Fig. 5j-o). Further, several cell-associated genomes were enriched. Hence, NCAPG might also play a role in promoting lung cancer cell proliferation. Therefore, NCAPG might promote NSCLC progression via immunosuppression, which was in accordance with previous studies.

3.8 Prognostic model

For a risk score, we further constructed a column line plot combining two independent prognostic factors, tumor stage, and NCAPG. We summed each score to obtain a final score, with a higher total score indicating a worse prognosis for the patient (Fig. 6a). We also plotted the calibration graphs, which

showed that the predictions at years, 3 years, and 5 years were closer to the actual situation, indicating that the NCAPG prognostic model has good accuracy in predicting the prognosis of NSCLC patients (Fig. 6b).

4 Discussion

NCAPG encodes a subunit of the chromosomal lectin complex, which is associated with the cell cycle and is responsible for chromosome cohesion and stabilization during mitosis and meiosis[21]. Previous studies have reported that NCAPG plays an important role in the development and progression of several cancers, including prostate, stomach, kidney, breast, and liver. Sun et al. showed that the expression of NCAPG was correlated with tumor progression in 135 gastric cancer and its adjacent tissues[22]. We performed bioinformatics analysis to identify whether NCAPG is a critical gene in the development of NSCLC. In addition, in recent years, increasing evidence has shown that an abnormal NCAPG expression is associated with tumor development. NCAPG expression was positively correlated with lymph node status, staging, and distant metastasis in a previous study[23]. However, the prognostic impact of NCAPG on immune infiltration in NSCLC should be further explored. Therefore, we performed a series of studies to determine further whether NCAPG promotes NSCLC progression.

Our immunohistochemical experiments revealed an elevated NCAPG expression in NSCLC, which was associated with a worse prognosis. Therefore, NCAPG can be an oncogene in NSCLC, which is important in improving the treatment of NSCLC. We assessed the diagnostic value of NCAPG expression using receiver operating characteristic curves ($p < 0.001$). Results showed that NCAPG had high sensitivity and specificity for diagnosing NSCLC. Multifactorial Cox regression analysis revealed that NCAPG expression was an independent prognostic factor for patients with NSCLC. Further, patients with a high NCAPG expression had a poorer prognosis than those with a low NCAPG expression according to the survival curves.

Furthermore, our study showed different sets of immune markers and the degree of immune infiltration correlated with NCAPG expression in NSCLC. Therefore, NCAPG may play a role in tumor immunology. In recent days, tumor immunotherapy has developed rapidly, and identifying factors that affect the state of the immune microenvironment may lead to the discovery of novel antitumor pathways (Fig. 7a). Moreover, the immune system was found to play an important role in cancer development and progression[24]. Identifying the impact of effective immune infiltration in NSCLC remains a major challenge[25]. The TME is an area of active research, and prognostic and diagnostic biomarkers or therapeutic targets must be explored (Fig. 7b). An accurate assessment of immune and prognostic biomarkers by evaluating immune infiltration can significantly help in the diagnosis and treatment of NSCLC[26]. The TME is significantly modified in LUAD and LUSC.

Moreover, we analyzed the composition of 22 immune subgroups in NSCLC using data from CIBERSORT[27]. Results showed that the NCAPG expression was negatively associated with tumor immune cells. B cells, CD8 T cells, CD4 memory T cells, neutrophils, macrophages, and dendritic cells (all

$p < 0.0001$) were negatively correlated[28]. Hence, NCAPG played an important role in tumor pathology for immune cell infiltration. T cells predominate, followed by B cells, macrophages, DCs, and natural killer cells, the main immune cell types in the TME in NSCLC[29]. Dendritic cells play an important role in activating anti-tumor T lymphocytes for specific antigen presentation[30]. As the most powerful specialized antigen-presenting cells, dendritic cells activate resting T cells, stimulate the initial immune response and perform a powerful immune surveillance function in the body (Fig. 7c). T cell infiltration is considered a predictor of better clinical outcomes. Low expression of NCAPG in NSCLC promotes T-cell activity and initiates an immune response against the tumor (Fig. 7d). Increasing interest in the role of tumor-infiltrating B cells in the TME. One study reported that increased B-cell infiltration in NSCLC is associated with a better prognosis[31]. Therefore, NCAPG could influence prognosis via immune activation and immune infiltration in patients with lung cancer.

GSEA further confirmed the possible involvement of NCAPG in immunosuppression in NSCLC. The NCAPG high expression not only decreases the survival rate of lung cancer cells but also induces apoptosis and blocks the S phase of the cell cycle by regulating N- and E-calmodulin[32]. Consistent with these findings, NCAPG was found to regulate NSCLC-related signaling pathways, DNA replication, cell cycle, and mismatch repair. GSEA further validated that NCAPG may promote tumorigenesis by regulating cell cycle, mismatch repair, and cellular senescence. Therefore, the overexpression of NCAPG in NSCLC is associated with poor prognosis by regulating the abovementioned pathways. Prognostic models suggest that NCAPG is a marker of prognosis in NSCLC patients.

5 Conclusion

We investigated the clinical impact of NCAPG on NSCLC and its role in tumor immunology. Results showed that the overexpression of NCAPG can be a prognostic biomarker for NSCSL and is a promising therapeutic target for NSCLC.

Abbreviations

NSCLC: Non-small cell lung cancer; NCAPG: Non-SMC condensin I complex subunit G ; LUAD: lung adenocarcinoma; LUSC: Lung squamous cell carcinoma TCGA: The Cancer Genome Atlas; TMA: Tissue Microarray; OS: Overall Survival; CIBERSORT: Cell type Identification by Estimating Relative Subsets of RNA Transcripts; TIMER 2.0: Tumor immune estimation resource, version 2; TME: Tumor Microenvironment; GSEA, gene set enrichment analysis; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes

Declarations

Ethics approval and consent to participate:

The present study was approved by the Research Ethics Committee of The Affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University.

Consent for publication:

All authors consent to the publication of this study.

Availability of data and materials:

We obtained a tissue microarray (TMA) (HlugC120PT01) from Superbiotek (Shanghai, China), which consisted of 140 non-small cell lung cancer tissue samples and 10 normal lung tissues. All patients had undergone lung cancer resection in 2005. All patients (112 men and 28 women) had access to complete clinical information.

Conflict of interests:

The authors have declared no competing interests.

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Authors' contributions:

This subject and manuscript were designed and written by Yong Zhou and Yongfei Fan.

Yong Zhou completed the experiment.

Ming Lou analyzed data compilation.

Xiaoshuang Liu was responsible for the literature search to improve the project and revise the manuscript. All authors reviewed the manuscript and approved the manuscript for publication.

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Compliance with Ethical Standards

Disclosure of potential conflicts of interest:

All the authors declare that they have no competing interests.

Research involving Human Participants and/or Animals:

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research.

Informed consent:

All patients or their family members provided written informed consent.

Disclosure:

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Availability of data and materials:

The data that support the findings of this study are openly available in The Cancer Genome Atlas (TCGA) data portal (<https://tcga-data.nci.nih.gov/tcga/>) and Gene Expression Profiling Interactive Analysis (GEPIA) (<http://gepia.cancer-pku.cn/index.html>), Tumor immune estimation resource (TIMER) database (<https://cistrome.shinyapps.io/timer/>). The rest of the data are available from the corresponding author on reasonable request.

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Tables

Table 1 Correlation between NCAPG expression and clinicopathologic features in patients With non-small cell lung cancer.

Characteristic	Low	High	P value
Age, y			
≤60	32 (22.9%)	34 (24.3%)	
>60	38 (27.1%)	36 (25.7%)	0.866
Sex			
Male	31 (22.1%)	33 (23.6%)	
Female	39 (27.9%)	37 (26.4%)	0.865
Location			
Left	57 (40.7%)	55 (39.3%)	
Right	13 (9.3%)	15 (10.7%)	0.833
Pathologic type			
Squamous cell carcinoma	42 (30%)	38 (27.1%)	
Adenocarcinoma	28 (20%)	32 (22.9%)	0.608
Tumor stage			
1	18 (12.9%)	10 (7.1%)	
2–3	52 (37.1%)	60 (42.9%)	0.139
Node stage			
0	43 (30.7%)	33 (23.6%)	
1–2	27 (19.3%)	37 (26.4%)	0.127
NSCLC stage			
I	49 (35%)	24 (17.1%)	
II–III	21 (15%)	46 (32.9%)	< 0.001
Os event			
Live	45 (32.1%)	28 (20%)	
Dead	25 (17.9%)	42 (30%)	0.007

Table 2 Univariate and multivariate Cox regression analyses of various prognostic parameters in patients with LUAD

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age	140	0.867 (0.532-1.411)	0.565		
Sex	140	0.889 (0.482-1.640)	0.706		
T	140	1.070 (0.583-1.963)	0.827	0.913(0.451-1.845)	0.799
N	140	1.703 (1.040-2.787)	0.034	1.378(0.717-2.646)	0.336
Clinical stage	140	1.809 (1.102-2.967)	0.019	1.129 (0.546-2.336)	0.743
NCAPG	140	2.300 (1.399-3.781)	<0.001	2.099 (1.218-3.619)	0.008

T for extent of the primary tumor; N for involvement of lymph nodes

Figures

Figure 1

NCAPG protein expression in 140 non-small cell lung cancer (NSCLC) tissues. (a) No staining, (b) weak staining, (c) moderate staining, (d) strong staining

Figure 2

a,b,c NCAPG expression was significantly higher in NSCLC, lung squamous cell carcinoma (LUSC), and lung adenocarcinoma (LUAD) than in paraneoplastic tissues ($p < 0.001$) d ROC curves of NCAPG expression in NSCLC. e,f The increase in NCAPG is associated with advanced clinical TNM and Os event. g,h,i Kaplan-Meier survival curves comparing high and low NCAPG expression. High NCAPG expression was associated with low overall survival, NSCLC ($p < 0.001$) LUSC ($p < 0.05$) LUAD ($p < 0.05$)

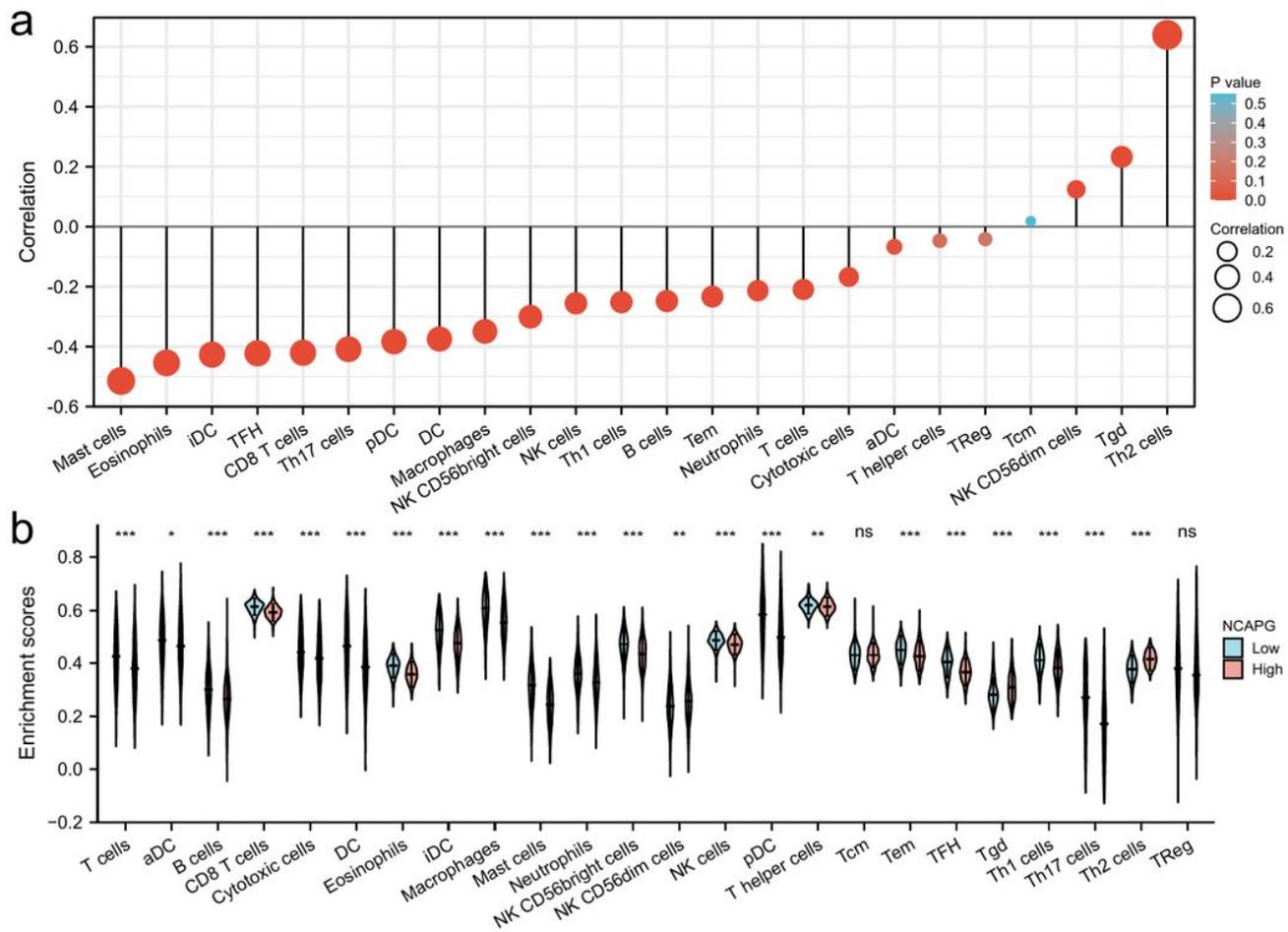


Figure 3

a 22 immune cells correlated with NSCLC. b T cells, CD8T cells, dendritic cells, macrophages, mast cells, NK cells and resting NK cells were negatively correlated with NCA PG expression. ($p < 0.001$).

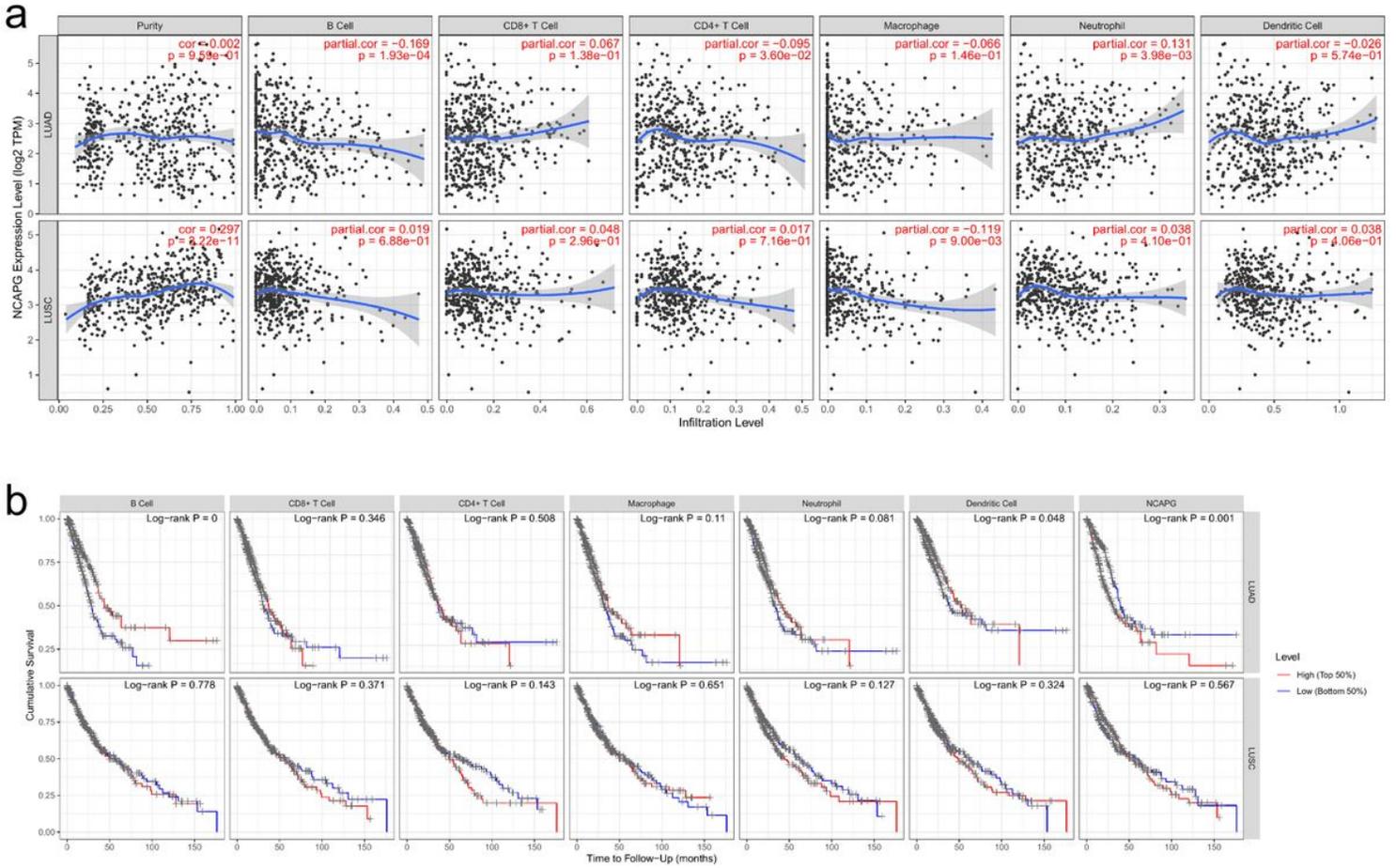


Figure 4

a Correlation of NCAPG expression levels with B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils and dendritic cells in NSCLC. b Six immune cells influence the cumulative survival of LUSC and LUAD.

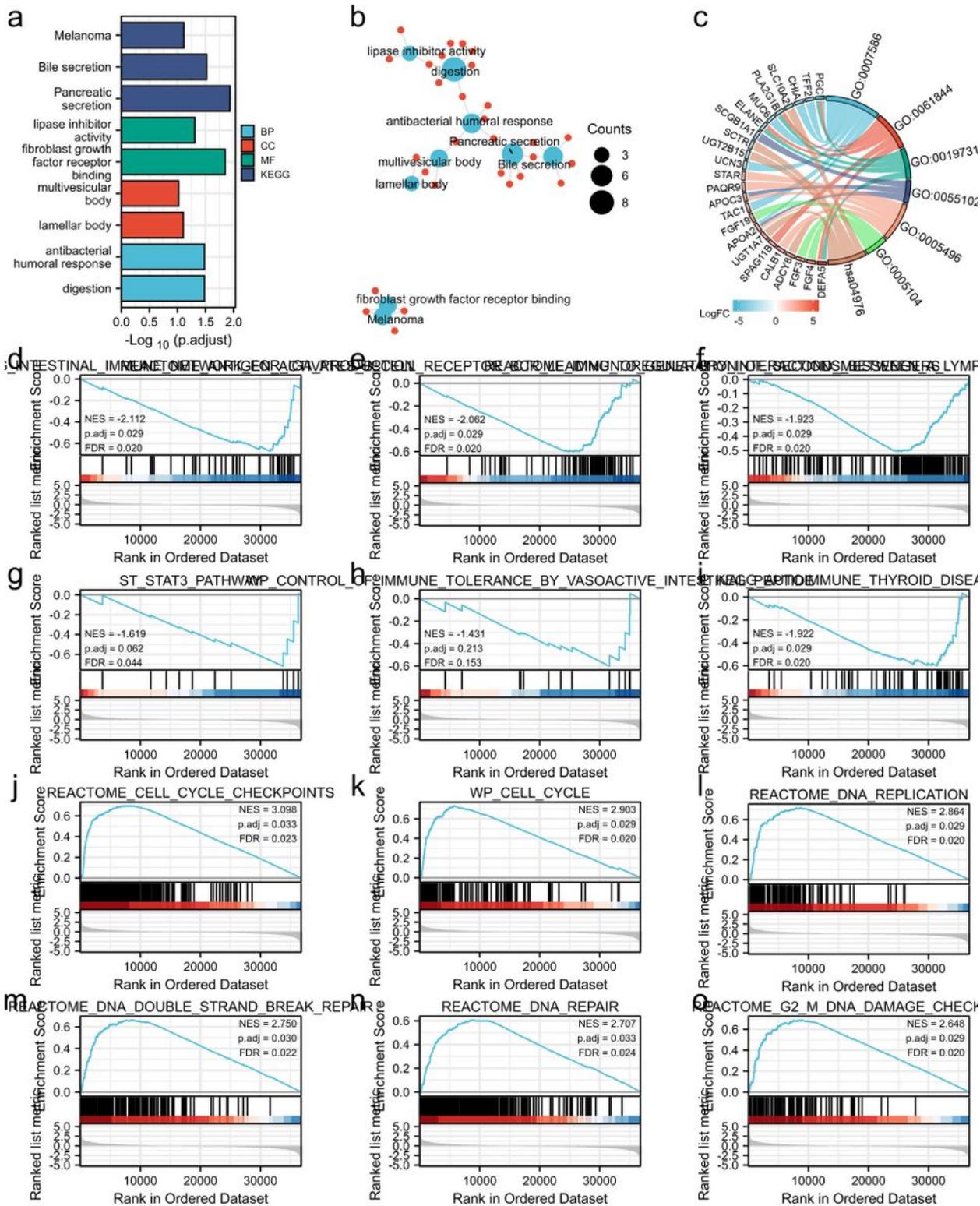
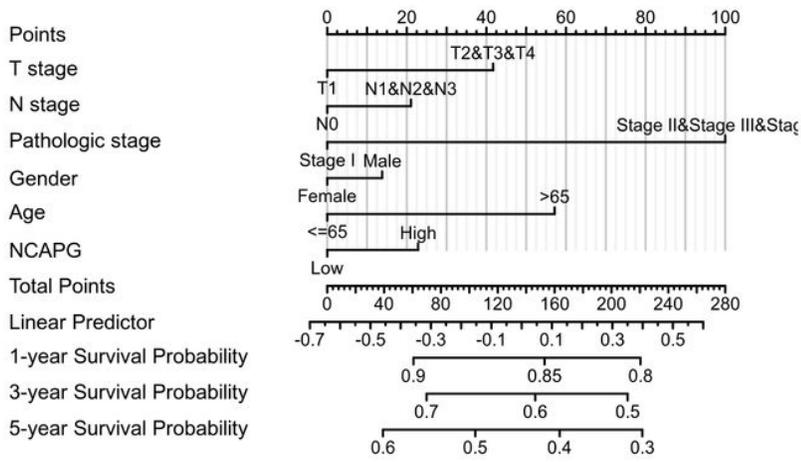


Figure 5

a The main mechanisms of NCAPG in NSCLC. b,c Genes significantly associated with NCAPG were shown. d-o GSEA results show differential enrichment between high S1DT1 expression and low S1DT1 expression.

a



b

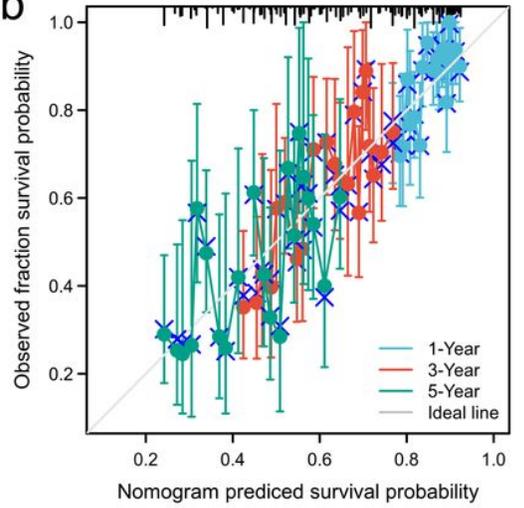


Figure 6

a Line graph of NSCLC prognostic model. b NSCLC prognostic model 1-year and 3-year 5-year calibration curves.

